

Proposing Drug Target(s) to Combat *Trypanosoma brucei* Infection

Taylor McGowen and Max Fontus

Prairie View A&M University

*Corresponding Author: Max Fontus, Texas Undergraduate Medical Academy,
Prairie View A&M University, P.O. Box 519 MS 2900, Prairie View, TX, 77446;
Phone: 936-261-3091; Email: mafontus@pvamu.edu*

Abstract

Background: Sub-Saharan African countries are plagued by an adaptable parasite, *Trypanosoma brucei*, *T.b.*, with a very high iron dependency. This protozoan parasite is the causative agent for Human African Trypanosomiasis (HAT), otherwise known as sleeping sickness. Two specific subspecies of interest include *T.b. gambiense* and *T. b. rhodesiense*. They lead to either a chronic or acute form of the fatal sleeping sickness disease, respectively. Few effective methods of treatment are available. The goal of this research project is to propose pharmaceutical drug target(s) to combat *T. brucei* infections. **Methods:** Studies analyzed the reaction pathways that take place during the parasite's life cycle, identified the natural and chemically produced superoxide radicals within the parasite, and also elucidated the differences in the parasite's life processes will allow for the derivation of a plausible methods to cease the infection cycle of the *T. brucei* parasite. The impact of these factors on preventing or reducing the virulence of the infection by *T. brucei* was assessed using computer-aided simulation software named *CLEMS*, Command-Line Electro-Metabolomics Software. We monitored the concentrations of oxygen, glucose and dihydroxyacetone phosphate, DHAP, given their importance for the survival of the parasite. Oxygen concentration peaked soon after the start of the simulation before achieving steady state with a concentration comparable to that of the mammalian environment simulated; glucose concentration was constant throughout the simulation again consistent with the environment where the parasite is evolving; finally, results showed a decrease in DHAP

concentration followed by steady state. Any process, which contributes to further decreasing DHAP concentration, is likely to be detrimental to the *T. brucei*. Other processes, such as superoxide radical degradation, will be considered in concert to the ones assessed in this study to develop suitable drug targets.

Introduction

Native to Sub Saharan African countries, the tsetse fly carries and transmits a fatal and often neglected tropical disease, Human African Trypanosomiasis, or HAT. This deadly disease affects at least 30 African countries.¹ The country with the highest infection rate is the Democratic Republic of Congo.² Of note, the number of reported cases of *Trypanosoma brucei gambiense* infections, which are chronic, is extremely higher than those of *T. b. rhodesiense*, which are acute. However, neither of these subspecies morphologically differs from one another.

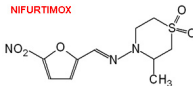
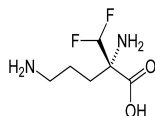
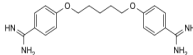
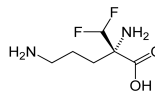
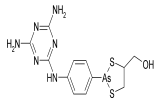
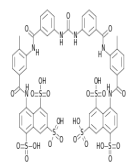
The transmission route and life cycles are also important. A tsetse fly will ingest the parasite where it is transported to the gut. The parasite is then in the procyclic form while inside the fly. Once the fly is infected, the insect becomes a vector of transmission for the disease until the end of its life. When the infected fly bites a human, it transfers the parasite directly into the human's bloodstream. Once in the bloodstream, the *T. brucei* parasite converts to the bloodstream form and circulates throughout the host's blood vessels and tissues.³ In this form, the parasite spreads to important mammalian fluids including: lymph and cerebrospinal fluid, along with those found in the placenta.² Symptoms include, but not limited to, fever, severe headaches, irritability, extreme fatigue, swollen lymph nodes and aching muscles and joints. The disease is diagnosed by finding the parasite in body fluid or tissue by microscopy.⁴

Current treatment options have certain limitations or harmful effects associated with each option. Certain drugs do not combat both the haemo-lymphatic and the meningo-encephalic stages of the sleeping sickness, all the while *T. brucei* has already begun to grow resistant to some treatments. Therefore, the need for a drug target that can

inhibit parasitic functions, preferably through distinct and parasite-specific reaction pathways inhibition, has increased in recent years.

Moreover, *T. brucei* possesses several unique characteristics that increase its survival rates. Naturally, every organism is comprised of multiple defense mechanisms. *T. brucei* is equipped with four superoxide dismutases—all requiring an iron, Fe, metal cofactor. Superoxide dismutases, SODs, are antioxidants used in the parasite's defense mechanism to rid itself of radicals via dismutation of superoxide radicals into hydrogen peroxide and molecular oxygen.⁵ This particular mechanism has been the target of many drug treatments (table 1).

Table 1 Existing Treatment Options for HAT: Available drug treatments for HAT along with possible side effects. Known structures are depicted as to facilitate future docking studies.

Drugs	Stage of Disease	Strain	Additional Info	Structure
NECT nifurtimox- eflornithine combination therapy	Central Nervous System Stage	Gambiense	Nifurtimox used in treat of Chagas disease	<p>NIFURTIMOX</p>  
Pentamidine	Circulator y Stage	Gambiense	Side effect: effects on the pancreas	
Eflornithine	Central Nervous System Stage	Gambiense	Side effect: Hematologic abnormalities, hearing loss, <u>Thrombocytopenia</u>	
Melarsoprol	Central Nervous System Stage	Rhodesiense	Arsenic derivative Side effect: Convulsions, fever, loss of consciousness, rashes, bloody stools, nausea, and vomiting. Encephalopathy	
Suramin	Circulator y Stage	Gambiense and Rhodesiense	Side effects: rashes, adrenal cortical damage, kidney damage	

T. brucei, receives all of its energy from glycolysis. This glycolysis cycle differs from other eukaryotes in that glycolysis is *T. brucei*'s sole ATP-producing process.⁶ The parasite metabolizes the glucose readily available in the host's bloodstream converting it into pyruvate. The first seven steps occur in the glycosome, a part of the cell with functions similar to peroxisomes.⁶ Other eukaryotes carry out the process in the cell's cytosol. This peculiarity makes *T. brucei*'s glycolysis mechanisms one of the main foci for drug target analysis.^{6,7} In addition, the parasite's glycolytic process can occur under both aerobic and anaerobic conditions. Some factors facilitating the parasite's adaptability include, yet not limited to: changes in coat surface and antigenic variation. The parasite exhibits antigenic variation, which is a switch in expression of variable surface glycoproteins, VSG, genes that encodes VSG coats.³ These thick coats protect the parasite from immune system attacks. *T. brucei* has multiple VSG genes, only expressing them one at a time.³ This particular adaptability contributes to the parasite's ability to bypass immunological responses and allows it to multiply in the mammalian bloodstream and eventually cross the blood-brain barrier. Unfortunately, minimal information is available on exact pathways during this switching process, and therefore much research is still needed. Current literature reveals that there are five existing treatment options for HAT (Table 1), and of the five some are either toxic to the host, too expensive for administration, or fails to address the infection in its entirety.

Chemotherapeutic drugs treat the disease by inducing oxidative stress (Table 1). A component of the most readily accepted treatment option, NECT, Nifurtimox leads to the formation of reactive oxygen species otherwise referred to as ROS.⁸ The drug can be orally administered and is readily absorbed throughout the body crossing the blood-brain barrier.⁹ Eflornithine, the other component of NECT combats the meningo-encephalic stage in *T. b. gambiense* infection but is ineffective against *T. b. rhodesiense*. The demand for a new pharmaceutical drug to combat sleeping sickness increases each day as the parasitic infection persists. To contribute to this search and study, computational modeling methods were used to assess impacts of reaction pathway disruptions. We simulated the glycolytic and the

superoxide radical degradation pathways of the parasite within the mammalian oxic environment.^{7,10} We monitored the concentrations of oxygen, glucose and DHAP as these represent three of the most important compounds that *T. brucei* relies on for survival.

Materials and Methods

Mathematically derived models of reaction pathways were developed and utilized for computer simulation. Reaction kinetics data was obtained through extensive literature review. The parasites' dire need for iron and the occurrence of free radicals due to the superoxide radical degradation pathway are pertinent to the discovery of the pharmaceutical drug target to annihilate the parasite without harming the hosts. The superoxide radical degradation pathway acts like the electron transport chain, severing oxygen molecules resulting in extremely reactive oxygen radicals. Manipulating a reaction's pathway to produce reactive oxygen radicals would in turn cause the parasite to self-destruct. Chokepoints, which are metabolites at the end of a metabolic process used as in other reactions pathways, constitute a major aspect in developing an improved drug target. Thus, they were followed closely throughout the simulation. Enzymatic activities of the multiple biochemical and electrophysiological species of the parasite were accounted for with specific Michaelis-Menten parameters. The formula:

$$h = \frac{VKY}{AK(K + c + c') + (A\theta cc')}$$

provides a four-state model for a facilitated diffusion carrier. In the above equation, h is the parameter that quantifies how fast the species can navigate the membrane, V is the maximum rate of exchange of species between compartments, K is the Michaelis-Menten constant describing transport properties between compartments, c and c' are respective concentrations, and θ is the symmetry index between compartments. The value for θ can vary between the integers 0 and 1.¹

Reactions that occur in the same cellular region or share some common species were classified, organized, and their kinetic values were computed. In addition, reactions were designated as either slow or fast, i.e. equilibrated. In the glycolysis pathway, 41 slow reactions that are taking place in three separate cell compartments were recorded and analyzed. The ATP-producing process that converts 2 moles of glucose into 2 moles of pyruvate is extremely intricate. Without the proper transportation of every compound and element, *T. brucei* suffers. Hence, proper identification of the location of each step in the process proves pertinent to calculations. The transportation of charged and rather large metabolites across an impermeable membrane dictates the usage of transporters. The transport of species against their gradients indicates active diffusion, which was modeled also in the simulation. The reactions were written in accordance to the mechanisms of the pathway. Parameters, such as maximum transport rate of a species from compartment to compartment, Michaelis-Menten constants, stoichiometry of reactions, and other specifications were calculated and calibrated, where information was incomplete, to give final concentrations of each species. Once all of the data was obtained and computed, the information was input into the *CLEMS* software (Max Fontus, Cypress, TX), where numerical datasets describing species concentrations at multiple time steps were produced. *CLEMS* generates quantitative data by converting biochemical and electrophysiological information into ordinary differential equations, which are then solved numerically.

All initial information was compiled^{1,7} in a master list containing species name, molecular weight, formal charge, and chemical symbol; each species was assigned a numerical value within the simulation (Table 2). Throughout the simulations, different environments and conditions can be easily assessed and according results investigated.

Table 2 Species Master List: These are the species involved in the five known important pathways of *T. brucei*. The associated charged of the species are also tabulated when reported in the literature or based on the pH level of the compartment where they are located in the course of a simulation. The charges in the parentheses are reflective of the fact that multiplicity of charges is possible based on pH

Species Name	Species number	Chemical Symbol/ formula	Charge	MW (g/mol)
Dihydroxyacetone phosphate (DHAP)	1	$C_3H_7O_6P$	0(-2)	170.06
Glucose	2	$C_6H_{12}O_6$	0	180.16
Glucose 6 phosphate (Glc-6-P)	3	$C_6H_{13}O_9P$	0(-2)	260.16
Fructose 6 phosphate (Fru-6-P)	4	$C_6H_{13}O_9P$	0(-2)	259.81
Fructose 1,6-bisphosphate (Fru-1,6-BP)	5	$C_6H_{14}O_{12}P_2$	0(-4)	340.116
Glyceraldehyde 3-phosphate (GA-3-P)	6	$C_3H_7O_6P$	0(-2)	170.058
1,3-bisphosphoglycerate (1,3-BPGA)	7	$C_3H_8O_{10}P_2$	0(-4)	266.03
3-Phosphoglycerate (3-PGA)	8	$C_3H_7O_7P$	0(-2)	186.06
2-Phosphoglycerate (2-PGA)	9	$C_3H_7O_7P$	0(-2)	186.06
Glycerol 3-Phosphate (Gly-3-P)	10	$C_3H_9O_6P$	0(-2)	172.074
Phosphoenolpyruvate (PEP)	11	$C_3H_4O_6P$	-1(-3)	167.034
Fructose 2,6-bisphosphate (F26BP)	12	$C_6H_{14}O_{12}P_2$	0(-4)	340.116
Hexokinase (HK)	13	Enzyme		
Phosphoglucose Isomerase (PGI)	14	Enzyme		
Phosphofructokinase (PFK)	15	Enzyme		
Aldolase (Ald)	16	Enzyme		
Triose Phosphate Isomerase (TIM)	17	Enzyme		
Phosphoglycerate Kinase (PGK)	18	Enzyme		

Table 2 Continued

Species Name	Species number	Chemical Symbol/ formula	Charge	MW (g/mol)
Glycerol 3-Phosphate Dehydrogenase (G3PDH)	19	Enzyme		
Glycerol Kinase (GK)	20	Enzyme		
Adenylate Kinase (AK)	21	Enzyme		
AMP-activated Protein Kinase (AMPK)	22	Enzyme		
Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH)	23	Enzyme		
Pyruvate Kinase	24	Enzyme		
Glycerol 3-Phosphate Oxidase (GPO)	25	Enzyme		
Enolase (ENO)	26	Enzyme		
Adenosine Triphosphate (ATP)	27	$C_{10}H_{16}N_5O_{13}P_3$	-4(-3)	507.18
Adenosine Diphosphate (ADP)	28	$C_{10}H_{15}N_5O_{10}P_2$	-3	427.201
Adenosine Monophosphate (AMP)	29	$C_{10}H_{14}N_5O_7P$	-2	347.22
Cyclic Adenosine Monophosphate (cAMP)	30	$C_{10}H_{12}N_5O_6P$	-1	329.206
Hydrogen Gas, H_2	31	H_2	0	2.016
Proton, H^+	32	H^+	+1	1.008
Sodium ion, Na^+	33	Na^+	+1	22.990
Potassium ion, K^+	34	K^+	+1	39.098
Chlorine ion, Cl^-	35	Cl^-	-1	35.453
Oxygen gas, O_2	36	O_2	0	31.998
Water, H_2O	37	H_2O	0	18.015
Nicotinamide Adenine Dinucleotide, NAD^+	38	$C_{21}H_{27}N_7O_{14}P_2$	-1	663.43
Reduced Nicotinamide Adenine Dinucleotide, $NADH$	39	$C_{21}H_{28}N_7O_{14}P_2$	-2	664.44
Pyruvate (Pyr)	40	$C_3H_4O_3$	-1	88.06
Glycerol (Gly)	41	$C_3H_8O_3$	0	92.09
Inorganic Phosphate, P_i	42	PO_4	-1	82.16
Phosphoglycerate Mutase (PGM)	43	Enzyme		
Ald-FBP	44			

Table 2 Continued

Species Name	Species number	Chemical Symbol/ formula	Charge	MW (g/mol)
Ald-DHAP	45			
END	46			
G3PDH-NAD	47			
EGN	48			
ESTE	49			
PAP	50			
ATP-PyK	51			
GAG	52			
ATP-GK	53			
PHD	54			
PHA	55			
PAMBG	56			
ATP-PGK	57			
PAMF	58			
ADP-PFK	59			
ESTPi	60			
HGAM	61			
ADP-HK	62			
ATP-HK	63			
ATP-PFK	64			
G3PDH-NADH	65			
ADP-GK	66			
PFK _{na}	67			
ADP-PGK	68			
ADP-PyK	69			
GAPDH-GAP	70			
PHA*	71			
Nicotinamide adenine dinucleotide phosphate (NADP ⁺)	72	C ₂₁ H ₂₉ N ₇ O ₁₇ P ₃	-3	744.413
Reduced Nicotinamide adenine dinucleotide phosphate (NADPH)	73	C ₂₁ H ₂₉ N ₇ O ₁₇ P ₃	-4	
6-Phosphogluconolactone (6- PGL)	74		0	
6-Phosphogluconate (6-PG)	75		0	
Ribulose 5-phosphate (Rul-5- P)	76	C ₅ H ₁₁ O ₈ P	+1	230.11
Ribose 5-phosphate	77	C ₅ H ₁₁ O ₈ P	+1	230.11
Carbon dioxide (CO ₂)	78	CO ₂	0	44.01

Table 2 Continued

Species Name	Species number	Chemical Symbol/ formula	Charge	MW (g/mol)
Trypanothione disulfide (TS ₂)	79	C ₂₇ H ₄₇ N ₉ O ₁₀ S ₂	0	721.84
Trypanothione [T(SH) ₂]	80	C ₂₇ H ₄₉ N ₉ O ₁₀ S ₂	-1	723.86
Ribose (Rib)	81	C ₅ H ₁₀ O ₅	+2	150.13
Superoxide	82	O ₂ ⁻¹	-1	32
Hydron, H ⁺	83	H ⁺	+1	1.008
Hydrogen peroxide	84	H ₂ O ₂	0	3.015
Oxygen	85	O ₂	0	16
Iron Superoxide Dismutase	86	Enzyme		
Superoxide Dismutase	87	Enzyme		

Results

Many species' concentrations oscillate until equilibrium is achieved. It can be observed that in glycolysis, the concentration of oxygen increases until it peaks, then decreases until steady state is achieved (figure 1). Oxygen is available in all compartments of the cell at all times, which would explain the major rise in the concentration until equilibrium is reached. Glucose concentration was constant, which is consistent with the fact that the mammalian host environment has a steady glucose concentration so that the parasite has a continuous source of metabolic fuel.

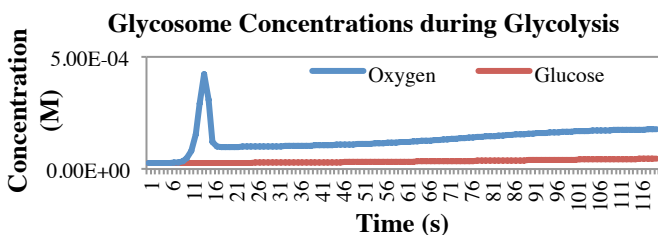


Figure 1 Concentrations of Oxygen and Glucose in the glycosome during a simulated glycolysis taking place in the mammalian bloodstream. These numbers were comparable to that of Navid^{1,7}

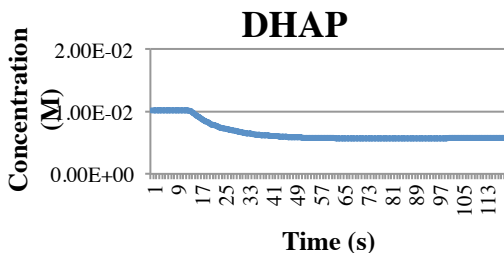


Figure 2 Concentration of DHAP in the glycosome during glycolysis where the conditions are the same as figure 1

The behavior of DHAP was monitored, given its importance in the anoxic, as well as anoxic, developmental conditions for the parasite. The obtained behavior of DHAP differed slightly from other factors. It started out almost constant; it then decreased until the concentration reached equilibrium between $5.00\text{e-}3$ and $6.00\text{e-}3$ M (figure 2). Any process contributing to further decrease in the concentration of DHAP would likely prove to be detrimental to the parasite.

Separate trials were run to validate the software by comparing its results to experimental historical data. Glycolysis was simulated by itself, but later on simulations involving glycolysis and the superoxide degradation pathway were done (Results not shown). This was designed to address the intertwining network of pathways *T. brucei* utilized to survive in multiple environments.

Conclusions

Glycolysis and superoxide radical degradation pathways were simulated in a computational environment based upon data from published literature. Given the importance of glycolysis, the parasite's sole ATP production process, and the superoxide radical degradation pathway, a chokepoint related to both pathways is believed to present and would be an ideal options for pharmaceutical drug target(s). This putative data confirms that inhibition of

glycolysis would lead to the destruction of *T. brucei*. Examination of additional chokepoints specific to each pathway is underway.

SODs are a major component of the defense mechanism in *T. brucei*. Analysis of the biochemical and molecular properties of mechanisms similar to the superoxide degradation pathway within the parasite is expected to provide better insights and holds promise for improved treatment intervention.

Unfortunately, HAT is a neglected disease in that it is not studied as much and subsequent studies tend to be unorganized. Specifics about the parasite in both life stages are not readily available and thusly cannot be used as part of the master input file, leading to an obvious limitation. However, having a fully functional and consistent electrophysiological model for *T. brucei*'s pathways and metabolism is of great significance. The parasite's high adaptability provides many such pathways and characterizes its greatest defense mechanism. Therefore, modeling other pertinent pathways taking place in *T. brucei* will expand and improve results from this project. With this tool, proposed mechanism(s) can be simulated for relevance. Once a mechanism is assessed, a target can be hypothesized. Drugs can be developed and simulated so that their capacity to eliminate or reduce infectivity, without negatively affecting the human host, can be studied well in advance of animal or human clinical trials.

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